Abstract and Introduction

My project focuses on characterizing different cyanobacteria in thrombolitic mats found on the island of Highborne Cay, Bahamas. Thrombolites are interesting ecosystems because of the ability of bacteria in these mats to remove carbon dioxide from the atmosphere and mineralize it as calcium carbonate. In the future they may be used as models to develop carbon sequestration technologies, which could be used as part of regenerative life systems in space. These thrombolitic communities are also significant because of their similarities to early communities of life on Earth. I targeted two cyanobacteria in my research, *Dichothrix spp.* and whatever black is, since they are believed to be important to carbon sequestration in these thrombolitic mats.

The goal of my summer research project was to molecularly identify these two cyanobacteria. DNA was isolated from each organism through mat dissections and DNA extractions. I ran Polymerase Chain Reactions (PCR) to amplify the 16S ribosomal RNA (rRNA) gene in each cyanobacteria. This specific gene is found in almost all bacteria and is highly conserved, meaning any changes in the sequence are most likely due to evolution. As a result, the 16S rRNA gene can be used for bacterial identification of different species based on the sequence of their 16S rRNA gene. Since the exact sequence of the *Dichothrix* gene was unknown, I designed different primers that flanked the gene based on the known sequences from other taxonomically similar cyanobacteria. Once the 16S rRNA gene was amplified, I cloned the gene into specialized *Escherichia coli* cells and sent the gene products for sequencing. Once the sequence is obtained, it will be added to a genetic database for future reference to and classification of other *Dichothrix sp.*

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Goals and Purpose

During my time at Kennedy Space Center, I worked in Dr. Jamie Foster's lab. My lab works microbialites, which are communities of microorganisms that adhere to each other or to a surface and are embedded in a extracellular polymeric substance (EPS) matrix. Microbialites are very similar in geologic structure to billion year old fossils of that may represent the first ecosystems that existed on Earth . Studying these modern day communities will give us knowledge about the early life on Earth and its evolution to the present day. These microbial mats also have the ability to take carbon dioxide out of their environment and sequester the carbon as calcium carbonate. The specific focus of the research is to identify the genetic mechanisms that these mats use to sequester carbon. This mechanism is important to study because of its potential applicability in human spaceflight and creating a closed, sustainable system in space. My job within this research was to target and characterize two photosynthetic specific microbes, known as cyanobacteria, which are believed to be important to carbon sequestration in the thrombolitic mats. Very little is known about the two types of cyanobacteria I researched, and in order to be able to identify these different species in future research, a method of identification had to be established for each.

The two types of cyanobacteria that I focused on, black and *Dichothrix*, were each identified and characterized by their 16S ribosomal RNA (rRNA) genetic sequence. This gene plays a significant role in phylogenetic studies because of its high level of conservation between different species. In other words, the sequence of the gene remains unchanged within a species over time. Any small changes in the sequence are due to evolution and can be used to

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differentiate between species. My ultimate goal was to obtain the 16S rRNA sequences for both the black cyanobacteria and *Dichothrix*.

To isolate the black cyanobacteria and *Dichothrix* from the mat samples, each was dissected out from the mat in into tubes. This ensured I extracted DNA from *Dichothrix* or the black cyanobacteria and not the various other microorganisms found in the mat. The genomic DNA from the samples was isolated from cell debris such as proteins and polysaccharides using a DNA extraction kit. The extracted DNA was quantified using a Picogreen Assay, which uses fluorescence to determine the very small amount of DNA present in each extraction. Using the DNA extracted from the *Dichothrix* and the black mats, the 16S rRNA gene was amplified using a technique called polymerase chain reaction (PCR). PCR is essentially a DNA copy machine - it can create thousands of copies of a specific gene from just a small amount of DNA. Once PCR products were successfully obtained, they were purified and cloned (inserted) into a plasmid vector, which is a circular piece of DNA. This vector (containing the 16S rRNA gene) was transformed into competent *E. coli* cells and streaked out on plates to grow overnight. The competent cells are designed to take up the vector, and as they divide, each new *E. coli* cell also contains a copy of the plasmid. *E. coli* divide rapidly, and after an overnight incubation, the cells containing the vector grew up into colonies on the plates. A few colonies were selected from the plates and incubated overnight in liquid media. A plasmid mini-prep was performed with the cells from the overnight incubation. This procedure breaks open the *E. coli* cells and purifies the plasmid DNA inside these cells that contain the target insert, which is the 16S rRNA gene. The plasmid DNA was then sent to the University of Florida for sequencing.

A large aspect of my project involved optimizing the PCR step to successfully obtain the 16S rRNA gene I was targeting. I spent time troubleshooting different reaction conditions and

component concentrations in order to optimize 16S rRNA amplification. I also designed specific

primers for the *Dichothrix* gene. Primers are short pieces of DNA that flank the gene on either

side so only the gene is copied and amplified. Because the sequence of the *Dichothrix* gene is

unknown, I designed the primers based off two other cyanobacteria phylogenetically similar to

Dichothrix: *Rivularia* and *Calothrix*. Mention how have been sent up for sequencing of the 16S

rRNA gene and how your research will help our lab in the future.

Characterization of black and *Dichothrix* cyanobacteria based on the 16S ribosomal RNA

gene sequence

Maya Ortega

Mentor: Dr. Jamie Foster

Kennedy Space Center

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The two types of cyanobacteria that I focused on, black and *Dichothrix*, were each identified and characterized by their 16S ribosomal RNA (rRNA) genetic sequence. This gene plays a significant role in phylogenetic studies because of its high level of conservation between different species. In other words, the mutation rate of the gene remains constant within a species over time. Species that have very similar 16S rRNA genetic sequences are phylogenetically very close to one another. As a result, this gene's sequence can be used to differentiate between

species. My ultimate goal was to obtain the 16S rRNA sequences for both the black cyanobacteria and *Dichothrix*.

To isolate the black cyanobacteria and *Dichothrix* from the mat samples, each was dissected out from the mat into tubes. This ensured I extracted DNA from *Dichothrix* or the black cyanobacteria and not the various other microorganisms found in the mat. The genomic DNA from the samples was isolated from cell debris such as proteins and polysaccharides using a DNA extraction kit. The extracted DNA was quantified using a Picogreen Assay, which uses fluorescence to determine the very small amount of DNA present in each extraction. Using the DNA extracted from the *Dichothrix* and the black mats, the 16S rRNA gene was amplified using a technique called polymerase chain reaction (PCR). PCR is essentially a DNA copy machine - it can create thousands of copies of a specific gene from just a small amount of DNA. Once PCR products were successfully obtained, they were purified and cloned (inserted) into a plasmid vector, which is a circular piece of DNA. This vector (containing the 16S rRNA gene) was transformed into competent *E. coli* cells and streaked out on plates to grow overnight. The competent cells are designed to take up the vector, and as they divide, each new *E. coli* cell also contains a copy of the plasmid. *E. coli* divide rapidly, and after an overnight incubation, the cells containing the vector grew up into colonies on the plates. A few colonies were selected from the plates and incubated overnight in liquid media. A plasmid mini-prep was performed with the cells from the overnight incubation. This procedure breaks open the *E. coli* cells and purifies the plasmid DNA inside these cells that contain the target insert, which is the 16S rRNA gene. The plasmid DNA was then sent to the University of Florida for sequencing.

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component concentrations in order to optimize 16S rRNA amplification. I also designed specific primers for the *Dichothrix* gene. Primers are short pieces of DNA that flank the gene on either side so only the gene is copied and amplified. Because the sequence of the *Dichothrix* gene is unknown, I designed the primers based off two other cyanobacteria phylogenetically similar to *Dichothrix*: *Rivularia* and *Calothrix*. My work troubleshooting and optimizing the processes required to sequence the 16S rRNA gene paved the way for others to perform the same procedures with ease and efficiency. I did obtain a number of partial sequences of the gene for both the black cyanobacteria and the *Dichothrix*, which will be published as part of a paper to identify these two cyanobacteria. The sequences will also be submitted to a genetic database that researchers around the world can access.

Impact of the MUST Internship on My Career Goals

The experience that I have had this summer has been incredibly influential on my background and knowledge as a molecular biologist. When I first began my internship in the Space Life Sciences Lab at Kennedy, I had very minimal lab experience and little understanding of what I wanted to do - and what options there were - in the field of molecular biology. My internship was heavily based on lab work, and I learned a great amount about working in a lab, including different procedures used with DNA and basic knowledge about chemicals, equipment, and safety. At the beginning of the summer my mentor walked me through all the different procedures necessary for my project, but after that I was on my own with respect to the actual lab work. It was very challenging for me and I definitely made my fair share of mistakes, but it truly gave me the opportunity to become familiar and comfortable with the procedures I was carrying out. Because I was repeating many of the procedures as part of the optimization process, I learned first-hand the purpose behind each step, and how the product is affected if a step is

changed. For example, I used bovine serum albumin (BSA) as a component of the PCR, and I found through trial and error that including more BSA generally promoted PCR amplification.

In my lab I worked with a post-doctoral research assistant, a doctorate candidate, a university professor, and a graduate student, and their experience from the many years they have spent working in different labs was very valuable to hear. As a scientist I benefited from the variety of knowledge they presented - each had different tips for helping procedures to run smoothly and useful pieces of information to help me better understand and optimize my experiments. I also appreciated being in the company of others who have been through master's and doctorate programs because it gave me a peek into what it takes to work towards each degree, and a better understanding of what I might like to pursue in my own future. I was especially interested in the M.D./Ph.D. programs they told me about, which is something I had never heard of but am now definitely interested in.

Aside from specific lab knowledge, I also learned much about my interests in molecular biology. The lab that I work in focuses on microbial communities, and analyzing gene expression and characteristic of the microbial mats. Working with DNA is exciting, and I love being able to visualize my results, but I found that I am not as enthusiastic about environmental biology as I am human and medical-related studies. Nevertheless, I certainly enjoyed working in a lab doing hands-on research, and I know that a career where I am in the lab or in the field doing work will best suit me.